MYICOLID COPEPODS AND MASS MORTALITY OF CULTURED HARD CLAMS (MERETRIX)

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ABSTRACT

Fifteen species of poecilostomatoid copepods are currently classified in the family Myicolidae. Except for Parostrincola lingulae, Humes & Boxshall reported from the mantle cavity of a brachiopod, the remaining 14 species of myicoids are known exclusively from the mantle cavities of marine bivalve molluscs. One of them, Pseudomyicola spinosus (Raffaele & Monticelli), is world-wide in distribution, it has been reported from 51 species of bivalve molluscs occurring in many parts of the tropical, subtropical and temperate waters. A general discussion on the anatomy of the myicoid copepods, particularly of the cephalic appendages, is given to illustrate the destructive nature of this family of copepods. The recent cases of mass mortality of cultured hard clams in the Far East are re-examined and Ostrincola hae Tanaka is shown to be the direct causative agent.

INTRODUCTION

It is not surprising to see that copepods are often dubbed the “Insects of the Sea”, because in terms of biomass, number of individuals, niche exploitation and distribution, the Copepodida constitute the most prevalent assemblages of marine, metazoan animals. Wiebe et al. (1992) went even further and stated that copepods are the most plentiful multicellular animals on earth, outnumbering the insects, which occur almost entirely on the land. According to Humes (1994), about 11,500 species of copepods are known. Though the diversity of copepods is not comparable to that of the insects, in the pelagic environment, copepods usually account for more than 70 % of the community, and it is not uncommon to find as many as 200,000 copepods per square meter in meiobenthos collections.

Although copepods are generally known as free-living aquatic animals, nearly half of them do engage in symbiotic mode of life. Huys & Boxshall (1991) stated that “copepods are parasitic on virtually every phylum of animals from sponges and coelenterates to vertebrates including mammals, and they enter into a variety of commensal or other associations with a similar range of hosts”. In the case of symbiotic existence, the number of individual copepods on a single host can also be very large. For instance, Abdelhalim (1990) reported over 13,400 individuals of Ergasilus sieboldi Nordmann (Ergasilidae: Poecilostomatoida) from the gills of a single freshwater fish in England, and Humes (1973) collected 17,294 individuals of Collocherides astroboae Stock (Asterocheridae: Siphonostomatoida) from two basket stars in Madagascar.

Currently, more than 430 species of Copepoda are known to occur as symbionts of five classes of Mollusca, namely, Aplacophora, Polycladophora, Gastropoda, Bivalvia and Cephalopoda, with Bivalvia as the most preferred hosts carrying more than half of the reported copepod symbionts. A great majority of these bivalve-inhabiting copepods are found inside the mantle cavities of their hosts and are often called “associates” of bivalves (Tanaka 1961; Reddiah 1962; Pillai 1963; Humes 1988; Stock 1993). However,
from the fact that these copepods are found neither in the plankton samples nor in the sediment collections, they should be considered as obligatory symbionts, if not outright parasites. I shall discuss in the following the destructive nature of some of these obligatory symbionts.

**COPEPODS OF THE FAMILY MYICOLIDAE**

Ten orders of Copepoda are currently recognized, and six of them are known to have their species occurring in symbiotic relationships with other aquatic animals. They are Calanoida, Harpacticoida, Monstrilloida, Cyclopoida, Poecilostomatoida and Siphonostomatoida. The last two orders alone contain nearly 85% of the symbiotic copepods. Among the symbiotic copepods, poecilostome copepods utilize the widest range of hosts, including all major groups of marine animals (sponges, cnidarians, turbellarians, molluscs, sipunculans, echinurans, decapod crustaceans, echnodermes, fishes) as well as algae (Ho 1991).

The symbiotic copepods found in the mantle cavities of bivalves are mostly members of the order Poecilostomatoida. So far eight of the 47 families of poecilostome copepods are known to utilize marine bivalves as hosts; they are: Antheesiidae, Clausidiidae, Clausiidae, Erebosasteridae, Lichomolgidae, Myricolidae, Mytilicillidae and Sabelliophilidae. While most poecilostomes are ectoparasites living in the mantle cavities of their host, members of the Mytilicillidae are characteristically found in the host intestine, the most common one of this kind is *Mytilicola intestinalis* Steur, living inside the intestine of oysters and mussels (Cheng 1967).

Among the eight families of poecilostome copepods mentioned above, Myricolidae is the one which shows the highest host-specificity to the bivalve molluscs. Fifteen species are currently known in this family, and, except for *Parostrincola ingulae* Humes & Boxshall, which was reported from the mantle cavity of a brachiopod at Hong Kong, the remaining 14 species are known exclusively from the mantle cavities of marine bivalve molluscs. One of them, *Pseudomyicola spinosus* (Raffaele & Monticelli) is world-wide in distribution; it has been reported from 54 species of bivalve molluscs occurring in many parts of the tropical, subtropical and temperate waters (Ho 1992).

Since only 243 species of bivalves from a few selected places on earth have been examined for symbiotic copepods, the number of the species of mycolids and their bivalve hosts would be much larger than the ones mentioned above when more bivalves from more different parts of the world are examined. For instance, before 1990, the copepod symbionts of bivalves were unknown in Korea, but through the effort of my colleague, Dr. Il-Hoi Kim of Kangreung National University, 31 species of edible Korean bivalves were found to harbour symbiotic copepods with 28 of them carrying two species of mycolid copepods, either *Pseudomyicola spinosus* or *Ostrincola koe* Tanaka. The interesting part of this is that 13 of those 31 species of Korean bivalves constitute new host records (Kim, pers. comm.).

As shown in Fig. 1A and 2E, to a non-specialist, the appearance of the mycolids are little different from a typical free-living copepod. However, at closer examination under a compound microscope, one will find that the mycolids have a pair of raptorial antennae (Fig. 1F), a pair of piercing mandibles (Fig. 1H) and another pair of rasping maxillae (Fig. 1J). These three pairs of cephalic appendages are the telltale of the true role the mycolids play in their bivalve hosts, even though their swimming legs (Fig. 1K, Figs. 2A,B,C) may give investigators an implication that they are innocent plankters which happened to be visiting the mantle cavities of bivalve molluscs. According to Yoshikoshi & Ko (1974), the mycolids feed
on host's mucus; however, the possession of piercing and rasping type of oral appendages implies that they may take host's tissue in addition.

Figure 1. *Ostrincola koe* Tanaka. Female: A, habitus, dorsal; B, urosome, ventral; C, genital complex, dorsal; D, caudal ramus; E, antennule; F, antenna; G, labrum; H, mandible; I, maxillule; J, maxilla; K, leg 1. Scale bars 0.1 mm. (after Ho & Kim 1991).
Figure 2. Ostrincola koe Tanaka. Female: A, leg 2; B, leg 3; C, leg 4; D, leg 5. Male: E, habitus, dorsal; F, posterioventral corner of genital complex; G, maxilliped; H, leg 5. Scale bars 0.1 mm. (after Ho & Kim 1991).
MASS MORTALITY OF CULTURED HARD CLAMS IN ASIA

The farming of hard clams (Meretrix spp.) has been practised for several decades in Asia, particularly in Japan, Korea, Taiwan and China. Although the occurrence of hard clam mass mortality was known back in the 60’s, the cases were always unpublished and dismissed as the results of pollution and/or over stocking. No serious attempt was made in pursuing the true cause(s).

So far as I am aware, the first published report of the cause of hard clam mass mortality was the one dealing with the occurrence in 1972 in the Buan sea-farming area located on the west (Yellow Sea) coast of South Korea. A species of bacteria, Pseudomonas ichthyodermidis, was suspected to be the causative agent that led to the eruption of mass mortality (Yoo et al. 1975). Later, a series of mass mortalities of Meretrix lusoria occurred in Taiwan in the early 80’s were attributed to an infection of another species of bacteria, Vibrio parahaemolyticus (Yang et al. 1987). A third species of bacteria, Vibrio alginolyticus, was isolated from diseased hard clams found in the affected areas in southern Jiangsu (estuary of Yangtze River) of China and it was reported as the causative agent of an epidemic “acute enteritis” that led to the mass mortality (Yu et al. 1990).

According to Chan et al. (1989), Vibrio parahaemolyticus and Vibrio alginolyticus are isolated frequently from seafood in the markets of Asian countries. Moreover, according to Liston & Baross (1973), these bacteria are common and ubiquitously distributed in the coastal waters of the tropical and temperate zones. Then, the question is: How can these bacteria gain access to various visceral tissues of the cultured hard clams so as to inflict an acute vibriosis? The answer to this question was found in 1990 when I visited the East China Sea Fisheries Research Institute in Shanghai, China.

In 1988 and 1989, the mass mortality of cultured hard clams recurred with devastating results in China, causing losses of about 6 million U.S. dollars in 1989 alone (Yu et al. 1990). Although it was identified as the result of an epidemic of acute enteritis caused by Vibrio alginolyticus (Yu et al. 1990), Zheng et al. (1990) reported that clams in the affected areas were heavily infested with a species of Ergasilus, a parasitic copepod, and the occurrence of this parasite was closely related to the eruption of vibriosis. Since Ergasilus is strictly a parasite of fish, these two reports from China intrigued me to visit China to find out the true identity of the causative agent(s) of clam mass mortality.

In China the clams infested with copepods would exhibit abnormal behaviour by rising to the surface of mud flat. They were locally called “Fou-tou” (meaning floating head). My examination of the copepods recovered from the water taken from the mantle cavities of the “Fou-tou” clams revealed that what was called “Ergasilus sp.” by Zheng et al. (1990) is actually a myicolid copepod, Ostrincola koe Tanaka. It is clear from the above discussion on the cephalic appendages of the Myicolidae, that the presence of unusually large numbers (up to 30 per host) of O. koe in the mantle cavities of “Fou-tou” clams is certainly a stress to the host, not only due to the loss of a large amount of mucus to the copepod parasites but also due to constant irritation brought about by the attachment and reattachment of parasites to the host’s gills by the powerful, hook-like antennae (see Fig. 1F). With the heavy loss of mucus and injuries on the gills (through parasite’s hooking, piercing and rasping), the host clam’s natural barrier to bacterial invasion would have been damaged. No wonder, Zheng et al. (1990) reported that the clams infested by copepods were all heavily infected with bacteria.
Our knowledge of the parasitic copepod fauna of bivalves in Asia is still very poor. Whereas 19 species of copepods are known to be parasitic on 16 species of commercial bivalves in Japan, their presence in cultured bivalves in Korea was reported for the first time only four years ago (Ho & Kim 1991) and they are yet to be surveyed in China and Taiwan. This pathetic ignorance together with the free-living-looking of the myciclid copepods are perhaps the major reasons for identifying in the past the bacteria (Yoo et al. 1975; Yang et al. 1987; Yu et al. 1990) or larvae of trematodes (Yoo et al. 1975) and cestodes (Kuo et al. 1984) as the primary causative agent of clam mortality.

**MYCICOLID COPEPODS IN SOUTHEAST ASIA**

Nine species of *Ostrincola* are currently known. Three of these are found in bivalves occurring in Southeast Asia. They are: *Ostrincola portonoviensis* Reddiah in *Meretrix casta* (Chemnitz), *Sanguinolaria diphos* and *Meretrix meretrix* (Linnaeus) from Portonovo, India (Reddiah 1962) in *Mesodesma trigona* (Deshayes) from Kerala, India (Pillai 1963); *Ostrincola breviseti* Ho & Kim in *Saccostrea cucullata* (Born) from Penang, Malaysia (Ho & Kim 1990); and *Ostrincola humesi* Ho & Yoosukh in *S. cucullata* from Gulf of Thailand (Ho & Yoosukh 1994). Interestingly, two of the three known species were discovered only recently. Since many species of bivalves in many parts of Southeast Asia are yet to be examined for their copepod parasites, it will not be surprising if we find more new species of myciclids as we make an effort to search. Certainly, many more bivalves will be recorded as new hosts for copepod parasites.

Inasmuch as *Ostrincola koe* has been recognized as a potential causative agent of clam mass mortality, undoubtedly, it is to the interest of aquaculturists as well as malacologists in Southeast Asia to be able to distinguish between the local species of *Ostrincola* from *O. koe*. To facilitate this attempt, three sets of illustrations were prepared and shown in Fig. 3 through Fig. 5. The general appearance of *Ostrincola* is much like the one shown for *O. koe* in Fig. 1A for the female, and in Fig. 2E for the

Figure 3. The second antenna of four species of female *Ostrincola* found in Asia.
male. The major species differences are found in those three appendages as shown in Fig. 3 for the antennae, in Fig. 4 for the fifth leg and in Fig. 5 for the caudal rami. A consult with “Key to the species of Ostrincola” prepared recently by Ho & Yoosukh (1994) is highly recommended.

Symbiotic copepods living in the mantle cavities of bivalve molluscs are generally small, about 1 mm in length on average. Therefore, it is rather difficult for non-specialists to examine clams, mussels, or oysters for their copepod parasites. To make this task easy, a collection procedure and method of examination were prepared and given in Appendix 1. To check whether the copepods that you obtained from bivalves are Ostrincola, consult a key for the genera of the Myicolidae prepared by Ho & Kim (1992).

**Figure 4.** The fifth leg of four species of female Ostrincola found in Asia.

**Figure 5.** The caudal rami of four species of female Ostrincola found in Asia.
CONCLUSION

Disease problems are often encountered in culture of aquatic organisms, particularly when an intensive and/or extensive system of culture is employed. Snieszko (1974) used an equation $D = H + P + S^2$ to explain the relationships between disease (D), host (H), pathogen (P), and stress (S) in the outbreak of infectious disease of cultured fishes. But, I believe the same equation applies equally well to the mass mortality of cultured hard clams, where the factor $S = (stress)$ includes not only the environmental changes (like the presence of pollutants), but also the presence of symbiotic copepods with destructive nature like the mycolids.

The pathogens (P) like *Vibrio alginolyticus* and *Vibrio parahaemolyticus* are ever present and ubiquitous in coastal water, but, under the normal situation they are not pathogenic to the clam. However, once the factor of “stress” is augmented through the presence of large number of symbiotic copepods in the mantle cavities, the clams (H) are irritated, non-pathogenic bacteria (P) gain access to the weakened clams, then vibriosis erupts and ends up in clam mass mortality. How can this result in a mass mortality? Because a single over-wintered female *Ostrincola koe* can produce several thousand offspring in one breeding season (Ho & Zhang 1994).

Therefore, it is utterly important to conduct a survey of symbiotic copepods before development of a massive farming of marine bivalve molluscs. Although *Ostrincola koe* has been identified as a potential causative agent of clam mass mortality, it does not follow that other species of *Ostrincola* will not do the same thing.

REFERENCES


APPENDIX 1.
Methods for Collection and Examination of copepod parasites occurring in the mantle cavities of bivalve molluscs.

Collection of copepod parasites
1. Scrub and wash the shell to clean all epizoons and epiphytes.
2. Open the cleaned bivalve in a glass Petri dish (about 10 cm in diameter or larger, depending on the size of host).
3. Wash the opened bivalve in a 1000 ml or 2000 ml beaker half filled with sea water. Wash only the same species of bivalves in a given beaker.
4. After having opened and washed all bivalves of the same species, pour the “washing” in the beaker through a plankton net.
5. Examine under a dissection microscope in a Petri dish (with a little sea water; enough to cover the dish bottom) the “debris” is collected in the plankton net.
6. Carefully remove the copepods with a pipette and preserve them in 70 % alcohol. Make one change of alcohol 48 hours later.
7. Examine also the Petri dish in which the bivalve was opened. Sometimes one finds copepods in this dish.

Examination of copepod parasites
1. Fill a watch glass with lactic acid and transfer into it the preserved copepod parasites.
2. Leave the copepod parasites in lactic acid for 2 or 3 hours until becoming semi-transparent.
3. Place the “cleared” copepod in a depression slide with lactic acid, cover it with a cover slip and examine the preparation under a compound microscope. The copepod can be “rolled” to an ideal position for examination by gently pushing the edge of the cover slip.

Note:
To make an accurate identification of the copepod parasites, one needs to dissect apart the minute appendages for close examination, magnifying up to 2,000 times. It requires high manual dexterity and long hours of practice. Besides, a special “wooden slide” technique is required for carrying out the close examination. This special technique can be found in the following publication: